Increasing Yields Using Urea With Urease Inhibitors

Improves efficiency of foliar fertilization in field and growth room tests.

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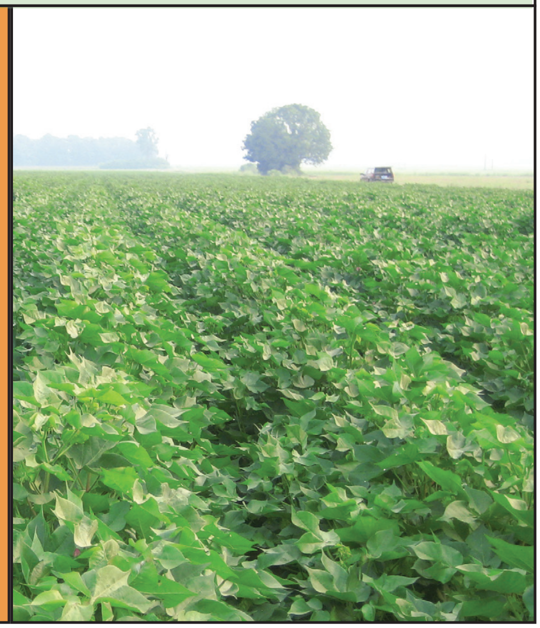
Summary: *Urea is the most recommended foliar nitrogen (N) source due to its relatively low toxicity, quick absorption and low cost. However, literature reports of yield increases with foliar urea application are inconsistent. The addition of a urease inhibitor may improve the use of foliar urea. Our study consisted of a growth chamber experiment using control, foliar urea, foliar urea + NBPT, and foliar NBPT. It also included a field experiment with: full recommended N soil rate with no foliar N application, a 75 percent N soil rate with no foliar application, a 75 percent of recommended N soil rate with two foliar urea applications, and a 75 percent of recommended N soil rate with two foliar urea + NBPT applications. In the growth room study the addition of NBPT to foliar urea inhibited urease activity and exhibited a trend for increased leaf urea content and improved cell membrane integrity. In the field study, the addition of NBPT to foliar urea resulted in an increase in seed-cotton yield. In conclusion, NBPT was effective in inhibiting cotton leaf urease, and in improving nitrogen use efficiency (NUE) and yield in field grown cotton.*



Foliar N application has been used as a supplement to meet cotton N requirements. Cotton root capacity for absorbing nutrients declines when the plants are developing fruit and, therefore, at this stage it is reasonable to supply N to plants by foliar application. Foliar application of N has the advantages of low cost and rapid response of the plant. It has the disadvantages of possible foliar burn, compatibility problems with other chemicals, and limitations on the amount of nutrient that can be applied. Many studies have been done testing the use of foliar urea in cotton; however, results for yield have been inconsistent. Once in the plant, urea is converted to ammonia by the enzyme urease and ammonia is incorporated to glutamate by the enzyme glutamine synthetase. In the available literature, it is still not clear whether leaf burn resulted from foliar urea application or is caused by toxic accumulation of urea or ammonia. In soybeans, foliar urea leaf burn is mainly associated with urea accumulation. However, to our knowledge, no research has been done in cotton. Use of a urease inhibitor with foliar urea application could be an effective method to help elucidate the fate of urea in cotton leaves. A well known urease inhibitor is N-(n-butyl) thiophosphoric triamide (NBPT) applied in the soil with urea. NBPT has been shown to have a high efficiency for inhibiting urease at low concentration in a wide variety of soils.

"Foliar urea increased seed-cotton yield"

Our preliminary data indicated that addition of NBPT to foliar urea increased cotton yield, with values significantly higher than urea alone. Furthermore, seed-cotton yield of NBPT + foliar urea treated plots, which received only 75 percent of the full recommended N rate, was statistically equivalent to plots that had 100 percent of the full recommended N rate. Thus, the use of urease inhibitor with foliar urea fertilization could have the potential of enhancing N assimilation in plant leaves, which could help improve foliar N management in crops.

Objectives

The main objectives of this study were to understand:

- Foliar urea assimilation in cotton plants.
- How the use of the urease inhibitor NBPT will affect the efficiency of foliar urea application.
- Do cotton leaves treated with urea suffer from toxicity of urea or ammonia?

With a better understanding of the physiological effects of foliar urea application and the use of a urease inhibitor, we expect to improve foliar N management in crops.

Growth Room Methodology

Planting. Cotton (Gossypium hirsutum L.) cultivar ST4554B2RF, was planted in 1.5 liter pots filled with soil from a representative cotton growing area in Marianna, AR. Pots were arranged in a large walk-in growth chamber.

Temperatures (day/night) were 30/20^{o}C, relative humidity 70 percent.

Fertilization rates (P_2O_5 and K_2O) were 45 and 73 kg/ha^{-1}, calculated using a soil volume of 1 ha and 0.15 m furrow slice. No soil N fertilization was applied in this experiment and pots were watered daily only with double deionized water.

Treatments consisted of:

- Untreated control with no foliar urea.
- Foliar urea.
- Foliar urea with NBPT.
- NBPT without urea.

Each foliar urea application was calculated to supply 11.2 kg of N per hectare. The treatment with urea plus NBPT was applied using the commercial fertilizer Agrotain (Agrotain Int. LLC) and the foliar NBPT rate was calculated based on reports that Agrotain contains 0.84 percent of NBPT. Treatments were applied at 8 a.m. four weeks after planting.

Field Methodology

Timing. The experiment was uniformly fertilized following preseason soil tests and state extension recommended rates, except for N, which was applied according to the treatments.

Treatments consisted of:

- Full recommended N soil rate with no foliar N application.
- 75 percent of recommended N soil rate with no foliar application.
- 75 percent of N soil rate with two foliar urea applications (at first flower and two weeks later).
- 75 percent of recommended N soil rate with two foliar urea plus NBPT applications (at first flower and two weeks later).

Each foliar urea application was calculated to supply 11.2 kg of N per hectare. The treatment with urea plus NBPT was applied using Agrotain. The full recommended N rate consisted of 125 kg N/ha^{-1}, and 93.7 kg N ha^{-1} was used for 75 percent of the recommended N rate treatment. Soil-applied N fertilization was sidedressed at planting and at the pinhead-square stage using urea.

Plots. The experiment was conducted using a plot size of 4 rows spaced 0.96 m apart by 15 m length.

Growth Room Results

A significant main treatment effect was observed for membrane leakage (P=0.0031) and MDA (P =0.0270). There was a significant decrease in membrane leakage and MDA for the NBPT treatment. For example, compared with the control, the NBPT treatment had a decrease of 20 percent (P= 0.0051) in membrane leakage and

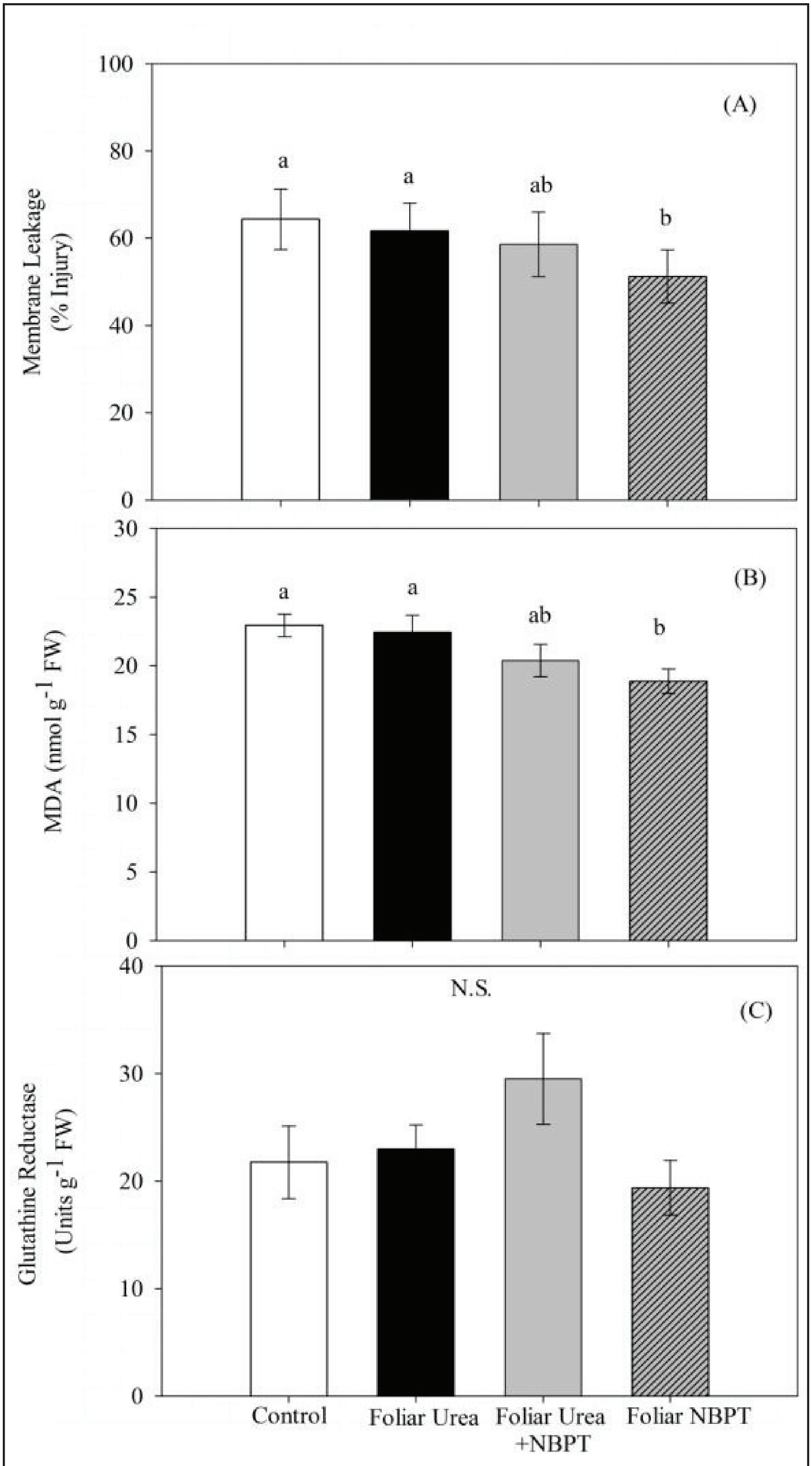


Figure 1. *Effect of foliar treatments on membrane leakage (A), MDA (B), and glutathione reductase (C) in cotton grown in growth room conditions. N.S. = not significant (P≤0.05).*

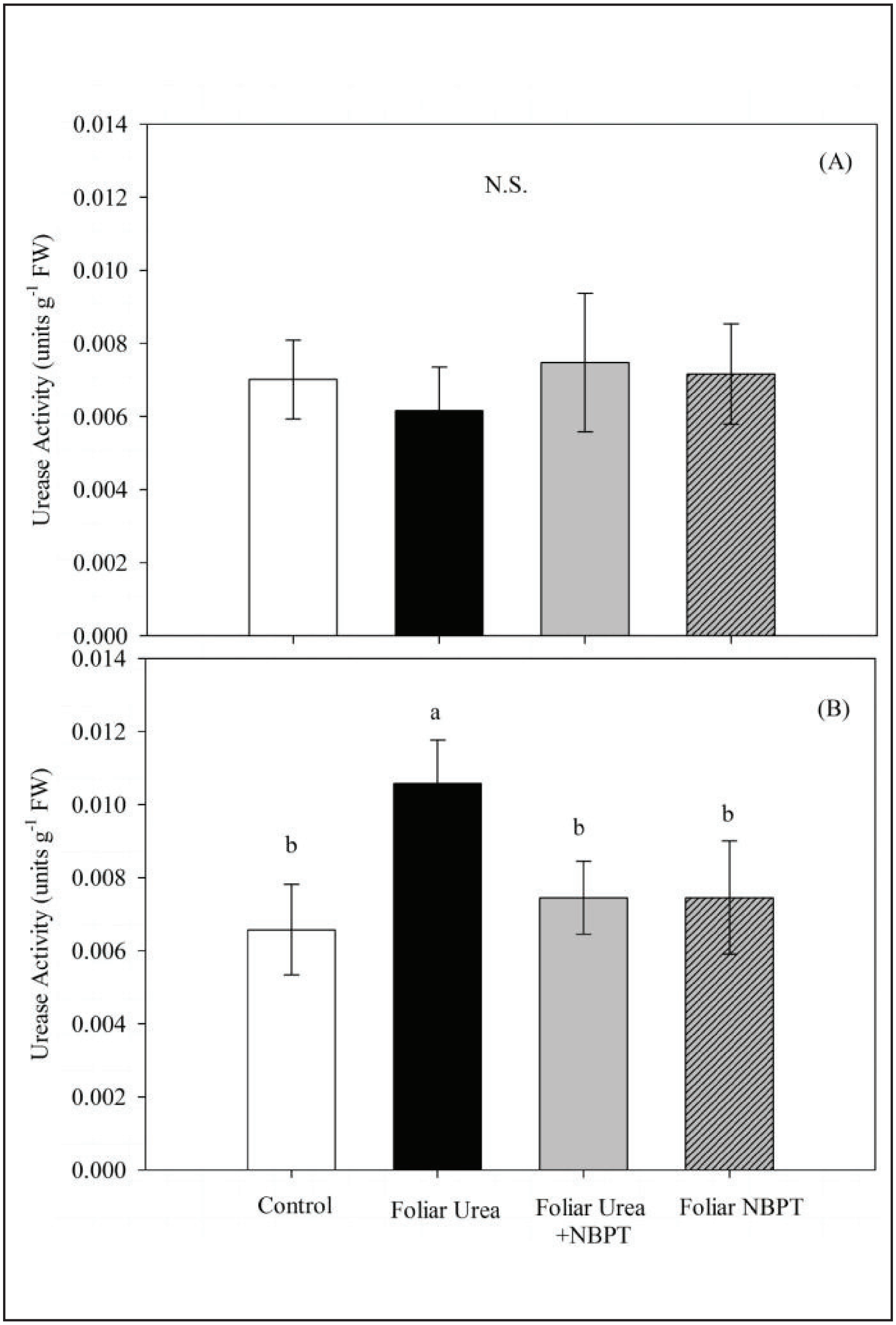


Figure 2: *Effect of foliar treatments on leaf urease activity measured at 2h (A) and 24 h (B) after application in cotton grown in growth room conditions. N.S. = not significant (P≤0.05).*

Table 1: Effect of foliar treatments on glutamine synthetase and leaf protein content (Growth Room Study).

Foliar Treatment	Glutamine Synthetase (mM glutamyl hydroxamate $g^{-1}FW\ hr^{-1}$)		Leaf Protein mg g^{-1} FW	
Control	0.070 ±	0.005	11.48 ±	0.21
Urea	0.064 ±	0.003	11.81 ±	0.18
Urea+NBPT	0.066 ±	0.004	11.37 ±	0.19
NBPT	0.063 ±	0.002	11.33 ±	0.21
P-Value	0.4354		0.1193	

18 percent (P=0.0070) in MDA content. The treatment Foliar Urea+NBPT (58.59±7.41 percent injury) had only a numerical decrease (P=0.827) in membrane leakage (Figure 1A) compared to the Foliar Urea treatment (61.65±6.38 percent injury). Similarly, data of MDA (Figure 1B) also indicated only a numerical (P=0.1761) decrease in the values of the Foliar Urea+NBPT (20.38±1.17 mmol g-1 FW) compared to the Foliar Urea (22.44±1.24 mmol g-1 FW) treatment.

Glutahione reductase data (Figure 1C) did not have any significant interaction or treatment effect (P=0.1191). The Foliar Urea+NBPT treatment had a numerical increase in GR values compared to the rest of the treatments; however, due to the high variability in the measurements, the data were not significantly different.

Data of urease activity (Figure 2) had a significant (P=0.0349) interaction effect between the parameters treatment and time of measurement. The analysis indicated that no significant treatment effect (P=0.7913) was observed in the measurements made at 2 h after foliar application (Figure 2A). However, measurements collected 24 h after foliar application (Figure 2B) showed a significant (P=0.0114) treatment effect, in which the foliar urea treatment exhibited significantly higher urease activity values than the rest of the treatments. In comparison to the Foliar Urea+NBPT (0.007±0.0001 units g-1 FW) treatment, the Foliar urea (0.011±0.0001 units g-1 FW) treatment had a 42 percent increase in urease activity (P=0.02335) when measurements were made 24 h after foliar application. Furthermore, the Foliar Urea+NBPT treatment did not exhibit increased urease activity; its values were not significantly different from the control treatment (P=0.4909).

Leaf urea content (Figure 3) measurement also indicated a significant (P=0.0382) interaction effect between the parameters treatment and time of measurement. In the measurement made 2 h after foliar application (Figure 3A) a significant treatment effect was observed (P=0.0200); however, the only statistical differences observed were when the Foliar NBPT treatment was compared with the treatments Foliar Urea (P0.0129) and Foliar Urea+NBPT

(P=0.0034). For the measurement made 24h after foliar application (Figure 3B), a significant treatment effect was also observed (P<0.0001). Compared to the control treatment a significant increase in leaf urea content was observed in the treatments Foliar Urea (P=0.0013) and Foliar Urea+NBPT (P=0.0006). In this case, the treatments Foliar Urea (3.15±0.18 mM g FW) and Foliar Urea +NBPT (3.57±0.18 mM g FW) and Foliar Urea+NBPT (3.57±0.44 mM g-1FW) had, respectively, a 48 percent and 68 percent increase in leaf urea content compared to the Control treatment (2.12±0.11 mM g-1FW). Significant differences were also observed when the Foliar NBPT treatments were compared with the treatments Foliar Urea (P=0.0003) and with Foliar Urea+NBPT (P=0.0002). On the other hand, comparative analysis of the Foliar Urea with Foliar Urea+NBPT (P=0.4780) and of the Control with Foliar Urea (P=0.5887) was not significant.

The data of GS (Table 1) and leaf protein (Table 1) content did not have any significant interaction or treatment effect. The treatment effect P values for GS and protein were, respectively, 0.4354 and 0.1193. Similarly, the measurement of photosynthesis (Table 2) and chlorophyll fluorescence (Table 2) had no statistical effect of interaction or treatment. In this case, the treatment effects P-values for photosynthesis and chlorophyll fluorescence were 0.1961 and 0.8531, respectively.

Field Results

A significant (P=0.0012) interaction effect between treatment and year of the experiment was observed in the data of seed-cotton yield. There was a significant (P=0.0029) treatment effect (Figure 4A) with the treatments 100 percent N Soil-No Foliar and 75 percent N Soil-Urea+NBPT Foliar exhibiting the highest yields. Significant differences were observed between the treatments 100 percent N Soil-No Foliar and 75 percent N Soil-No Foliar (P=0.0013), between 100 percent N Soil-No Foliar and 75 percent N Soil-Urea Foliar (P=0.00167), between 75 percent N Soil-No Foliar and 75 percent N Soil-Urea+NBPT Foliar (P=0.0017), and between 75 percent N Soil-Urea Foliar and 75 percent N Soil-Urea+NBPT Foliar (P=0.0221). No differences were observed between the treatments

Table 2: Effect of foliar treatments on leaf photosynthesis and chlorophyll fluorescence (Growth Room Study).

Foliar Treatment	Leaf Photosynthesis		Chlorophyll Fluorescence	
	µmol m^{-2} s^{-1}		Yield (Fv/Fm)	
Control	12.46 ±	0.60	708.06 ±	14.98
Urea	13.00 ±	0.47	703.98 ±	9.17
Urea+NBPT	13.36 ±	0.50	698.98 ±	6.64
NBPT	13.58 ±	0.34	702.65 ±	7.00
P-Value	0.1961		0.8531	

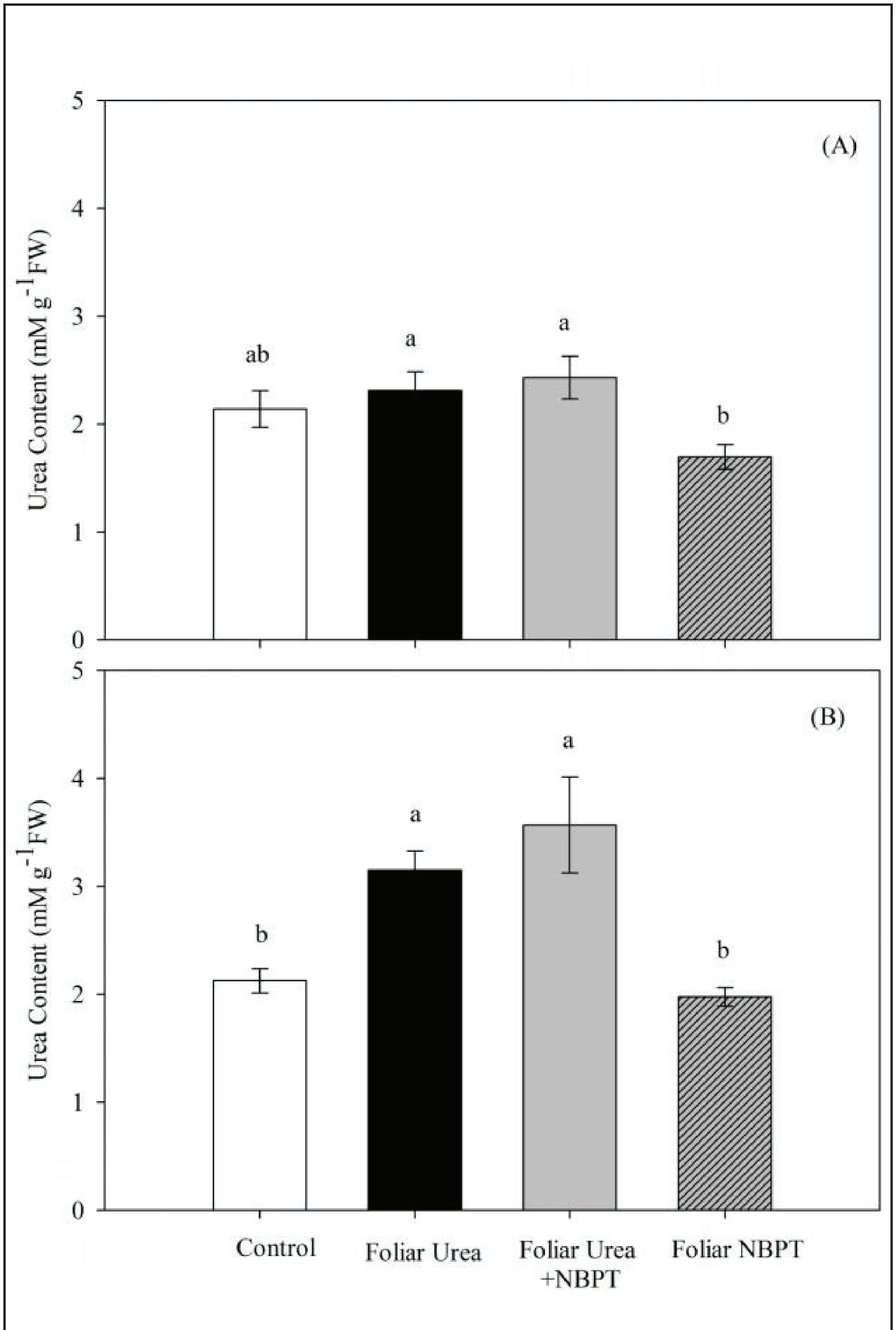


Figure 3: *Effect of foliar treatments on leaf urea content measured at 2h (A) and 24 h (B) after application in cotton grown in growth room conditions.*

100 percent N Soil-No Foliar and 75 percent N Soil-Urea+NBPT Foliar (P=0.8831), and between 75 percent N Soil-No Foliar and 75 percent N soil-Urea Foliar (P=0.1901). Comparative analysis of the treatments indicated that 75 percent N Soil-Urea+NBPT Foliar (1997.10±108.25 kg ha-1) exhibited a 20 percent and 12 percent increase in seed-cotton yield compared to the treatments 75 percent N Soil-No Foliar (1660.05±61.52 kg ha-1) and 75 percent N Soil-Urea Foliar (1776.60±62.68 kg ha-1), respectively. In 2010, (Figure 4B), the treatment effect on seed-cotton yield was not significant (P= 0.0951). Differences were expected between treatments 100 percent N Soil-No Foliar and 75 percent N Soil-No Foliar, but the comparison was not significant (P=0.1106).

There was a significant effect (P<.0001) for leaf burn in 2010 (Figure 5A). However, the comparative analysis only indicated that higher values of leaf burn occurred in the plots that received foliar urea application. No significant differences were observed between the treatment 75 percent N Soil-Urea Foliar and 75 percent N soil-Urea+NBPT Foliar (P=0.26639).

Measurement of leaf N (Figure 5B) and petiole nitrate (Figure 5C) content indicated no significant interaction or treatment effect. The P-values for the treatment effect were, respectively, 0.4197 and 0.2955 for leaf N and petiole nitrate data.

Our basic findings in the growth chamber study were:

- Application of only NBPT decreased membrane leakage and MDA
- Addition of NBPT-to-foliar-urea decreased urease activity measured at 24 h after application and had no effect in the measurements of GS, GR, protein, photosynthesis, and chlorophyll fluorescence.

Our basic findings in the field study were:

- Addition of NBPT to foliar urea resulted in a yield increase.
- Addition of NBPT to foliar urea application had no significant effect on leaf burn, leaf N, and petiole nitrate content.

Summary

In one published study, foliar urea application with the urease inhibitor phenylphosphorodiamde (PPD) has been reported to have a negative effect on soybean leaves. This study hypothesized that soybean leaf-tip injury caused by foliar urea application was attributed to ammonia formation from urea hydrolysis; however, they reported that the leaf necrosis was attributed to toxicity of urea rather than of ammonia. On the other hand, a later study with wheat did not observe any negative effect from NBPT with foliar-applied urea. In our study the negative effect of adding the urease inhibitor to foliar urea was not evident. We observed that addition of NBPT to foliar urea was effective in inhibiting leaf urease activity measured at 24 h after application. This mode of action of NBPT is carried by a binding and deactivation of the urease receptor for urea. The efficacy of NBPT in inhibiting urease in the soil is well documented. However, to our knowledge there is no report of NBPT effect on leaf urease activity. Since the addition of NBPT to foliar urea decreased urease activity it was expected that NBPT would result in increased leaf urea content. However,

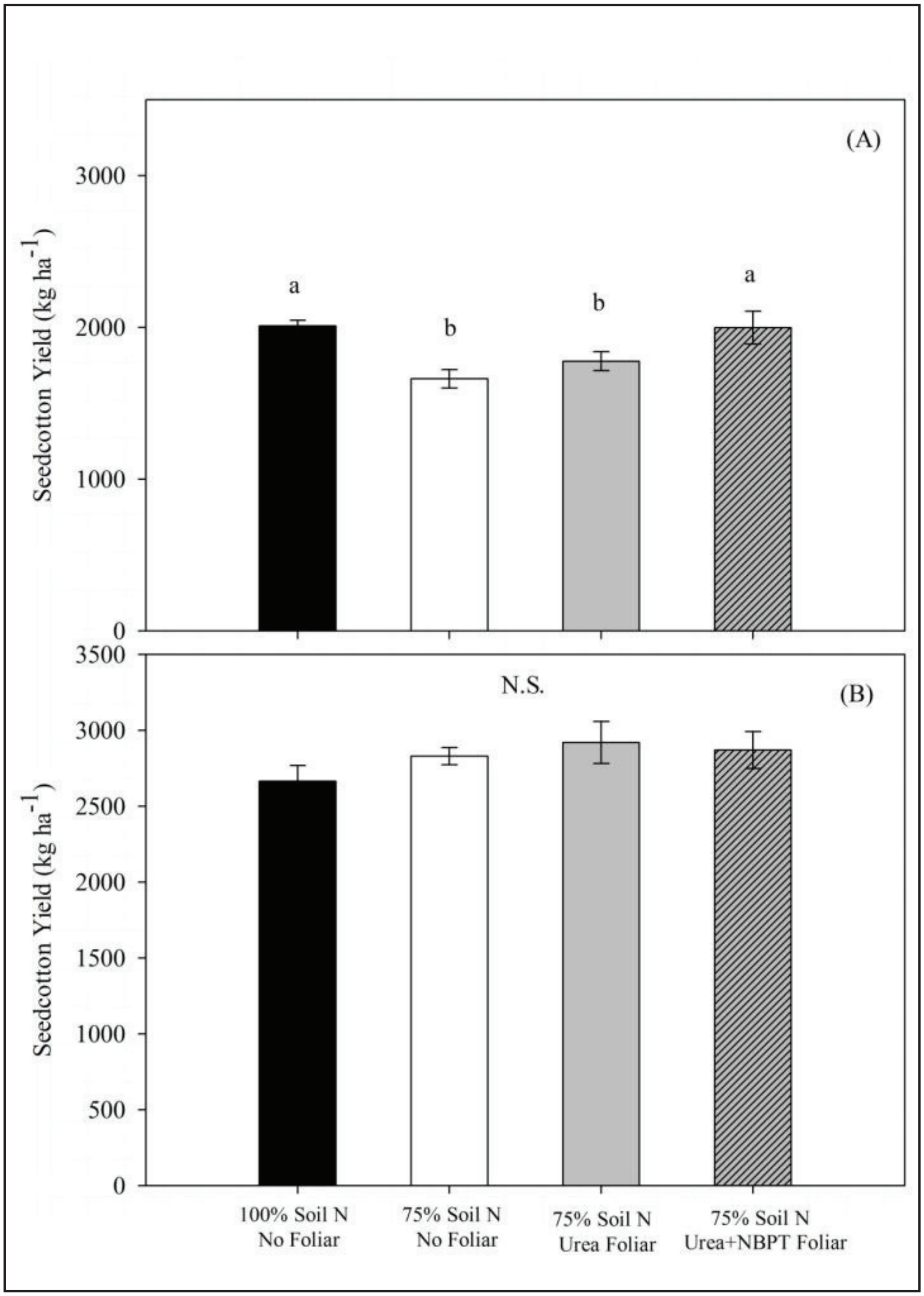


Figure 4: *Effect of foliar treatments on seedcotton yield of field grown . N.S. = not significant (P≤0.05).*

urea measurement collected at 24 h after treatment application showed no significant differences between the treatments Foliar Urea and Foliar Urea+NBPT. There was a numerical increase in leaf urea content with addition of NBPT, thus it is possible that a statistical difference could be detected if the measurements were done after the 24 h period.

The data of urease and urea in cotton indicated that the total hydrolization and assimilation of the foliar applied urea are not completed in the period of 24 h. The data of membrane leakage and MDA had identical results, indicating that application of Foliar NBPT improved the cell membrane integrity of cotton leaves. The treatment Foliar Urea+NBPT showed statistically equal values compared to the Foliar NBTP treatment; however, its values were not significantly different from the Foliar Urea treatment. The process involved in the role of NBPT on cell membrane integrity is not clear; however, since NBPT binds to Ni urease receptor sites, it is possible that NBPT has a Ni chelating effect in the plant. A 1992 study reported that Ni affected the cell plasma membrane properties and ATPase activity of rice plants. Another study reported Ni causing oxidative stress in a variety of plants, thus NBPT in the plant could be resulting in a protective mechanism against Ni. In this experiment, no evidence of a negative effect of urea and/or NBPT was observed in the measurements of GR, GS, protein, photosynthesis and chlorophyll fluorescence. However, it is possible that an effect of NBPT could occur in a measurement collected after the 24 h sampling, since a significant NBPT effect was observed in urease and membrane integrity data. Additional research is needed to address this hypothesis.

The yield data of the field experiment showed a significant interaction between treatment and year of the experiment. This indicated that the values of seed-cotton yield responded differently to foliar treatment applications, depending on the year of the experiment. We observed a significant seed-cotton yield increment with addition of NBPT to foliar urea. Addition of NBPT increased yield compared to application of foliar urea alone and it resulted in equivalent seed-cotton yield to the 100 percent N Soil application treatment. However, data on leaf burn, leaf N, and petiole nitrate content did not show any significant effect of the addition of NBPT to foliar urea application. The significant influence of NBPT on cotton yield could result from the NBPT effect on the inhibition of urease and improvements of cell membrane integrity indicated in the growth chamber study.

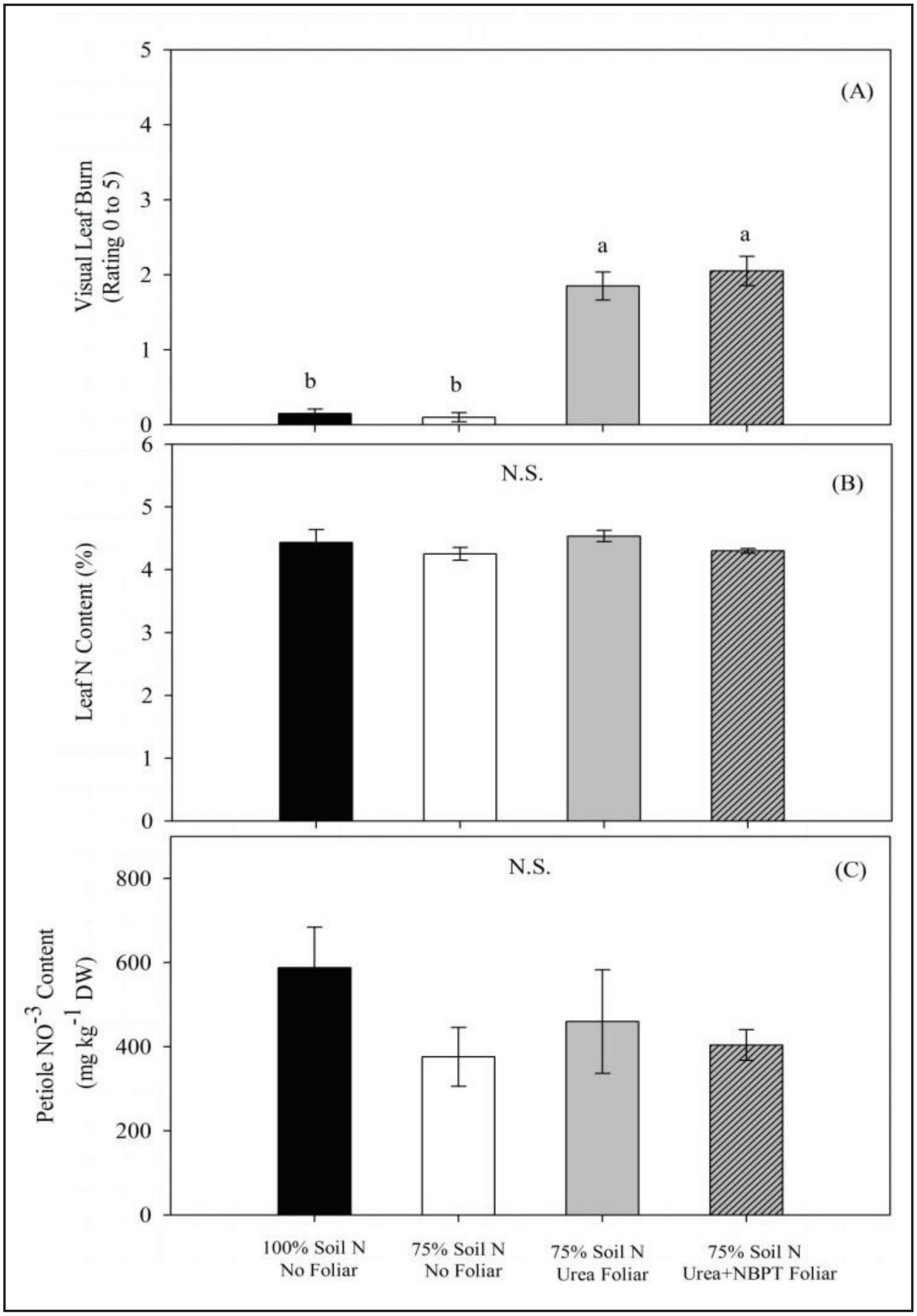


Figure 5: *Effect of foliar treatments on leaf burn (A), leaf N (B), and petiole nitrate (C) of a field grown cotton (2010). N.S. = not significant (P≤0.05).*

In conclusion, the use of NBPT to foliar urea application in the growth chamber study decreased urease acridity and showed trends for increasing leaf urea content and improving cell membrane integrity. In the field study, seed-cotton yield improvements were observed with the addition of NBPT to foliar urea.

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